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SINGLET OXYGEN ($^1\Delta_g$ O_2) REACTIVITY OF SOME PUTATIVE BIOLOGICAL PROTECTORS. Thomas A. Dahl, W. Robert Midden and Philip E. Hartman, Department of Biology and Department of Environmental Health Sciences, The Johns Hopkins University, Baltimore, MD 21218.

We compared by HPLC the relative abilities of some putative biological protectors to block oxidation of 2,5-bis(hydroxymethyl)furan (BHMF) in illuminated solutions containing the photosensitizer rose bengal and in the separated-surface-sensitizer (S-S-S)system of Midden and Wang (1983 J. Amer. Chem. Soc. 105:4129) involving pure $^1\Delta_g$ O_2. While L-histidine is a well-known quencher of $^1\Delta_g$ O_2 (Bellus 1979 Adv.Photochem. 11:105; Lindig and Rodgers 1981 Photochem. Photobiol. 33:627), free L-histidine is not a prevalent biomolecule. Carnosine (β-alanyl-L-histidine = CAR), however, is present in striated muscle in concentrations up to 20 mM (Crush 1970 Comp. Biochem. Physiol. 34:3). CAR quenches $^1\Delta_g$ O_2 at least 10% more effectively than does equimolar L-histidine both in solubilized sensitizer studies and in the S-S-S system. 2-Thiolhistidine and its fungal analog, ergothioneine (2-thiolhistidine betaine), and urate at 1 mM each block photosensitized BHMF oxidation using solubilized sensitizer better than does azide, but all three compounds fail to quench BHMF oxidation by $^1\Delta_g$ O_2 in the S-S-S system. Since BHMF oxidation is due almost exclusively to reaction with $^1\Delta_g$ O_2, urate and the two imidazole sulfhydryl compounds may be inhibiting oxidation induced by the solubilized photosensitizer by quenching the sensitizer excited state and thereby reducing the $^1\Delta_g$ O_2 concentration. Urea did not quench BHMF oxidation in either sytem. We conclude that some biomolecules afford protection against $^1\Delta_g$ O_2 and that distinctive mechanisms of protection may operate in individual cases; these include direct interception of $^1\Delta_g$ O_2 and prevention of $^1\Delta_g$ O_2 production by quenching excited state molecules. (Supported in part by grants ES03217 to P.E.H. and ES02300 to W.R.M.)

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MUTAGENICITY OF LEACHATES FROM A MODEL COAL PILE. C. B. Daniels, S. M. Baksi, J. H. Tuttle* and J. C. Means, Chesapeake Biological Laboratory, Solomons, MD

It is not uncommon to find coal stockpiled in massive amounts on the premises of industrial plants and utilities due to recent increases in coal consumption. Rainfall on these coal piles has been found to produce runoff containing PAH's of a mutagenic and carcinogenic nature. Increased stockpiling of coal may therefore serve to heighten the potential of generating mutagenic waste. Experiments were performed to assess the mutagenicity of leachates produced on simulated coal piles in a reversion assay, Ames test, and in a forward mutation assay with arabinose sensitive strains of Salmonella. Coal piles consisting of a low sulphur bituminous coal were constructed and rainfall events were simulated every 14 days. Leachates were passed through a series of five liquid-liquid extractions at pH2 and pH10. Acid and base fractions were combined, concentrated, brought to near dryness, weighed and suspended in DMSO. Extracts, 50-1000ug/plate, were tested for mutagenicity with the Ames test using TA98 and TA100 and in a forward mutation assay using Salmonella strain SV50. Extracts were pre-incubated with bacteria at 37°C for 20 min both in the presence and absence of metabolic activation (rat S9). Poured plates were incubated at 37°C for 48h (TA98 & 100) and 72h (SV50) before scoring mutants. Initial investigations showed extracts resulting from the first rainfall to be highly toxic. Dilutions of extracts from subsequent events, rains 2 & 3 and 4 & 5, showed the leachates to be mutagenic with a greater degree of activity noted in the presence of activation. Studies of these simulated coal piles suggest that stockpiling of coal may pose a mutagenic hazard to aquatic organisms exposed to the runoff.
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PEROXIDASE-MEDIATED METABOLIC ACTIVATION OF AMITROLE Diane L. Daston*, Robert S. Krauss*, Thomas E. Eling*, William J. Caspary NIEHS/NIH, RTP, NC 27709

The herbicide amitrole has been used to control poison ivy and poison oak and to manage weeds on crops. In 1959, amitrole was found to induce thyroid and liver tumors in rodents. A limited epidemiological study suggested that amitrole is carcinogenic to man, but the results were not conclusive. Amitrole did not induce mutations in the Salmonella reversion assay or in mouse lymphoma cells in either the presence or absence of S9. However, this compound did induce mutations and cell transformation in Syrian hamster embryo cells. These cells are metabolically active, suggesting that a metabolic pathway not present in the S9 may be responsible for its activity. When prostaglandin synthetase (PGS) was added to phenylbutazone, a substrate for peroxidase, oxygen was consumed. Addition of amitrole to this mixture caused an inhibition of oxygen uptake, suggesting that amitrole competes with phenylbutazone and is a reducing cofactor for the peroxidase. Microsomal PGS, in the presence of arachidonate or H_2O_2, catalyzed the covalent binding